510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE

A. 510(k) Number:

k060619

B. Purpose for Submission:

New device

C. Measurand:

Carbon dioxide

D. Type of Test:

Quantitative

E. Applicant:

Diazyme Laboratories

F. Proprietary and Established Names:

Diazyme Carbon Dioxide Enzymatic Assay Kit
Diazyme Carbon Dioxide Controls

G. Regulatory Information:

1. Regulation section:

   21 CFR 862.1160
   21 CFR 862.1660

2. Classification:

   Class II
   Class I, reserved

3. Product code:

   KHS
   JJX

4. Panel:

   Chemistry (75)

H. Intended Use:

1. Intended use(s):

   Diazyme Carbon Dioxide Enzymatic Assay is for the in vitro quantitative determination of carbon dioxide content in human serum or plasma.

   Diazyme Carbon Dioxide Controls are assayed QC materials for use in quantitative in vitro diagnostic determination of carbon dioxide in human serum and plasma. They are intended as reference samples for monitoring the Diazyme Carbon Dioxide Enzymatic Assay.

2. Indication(s) for use:

   Diazyme Carbon Dioxide Enzymatic Assay kit, in conjunction with Diazyme Carbon Dioxide Calibrators, are intended for the quantitative determination of carbon dioxide (CO_2) in serum and plasma.

   Diazyme Carbon Dioxide Enzymatic Assay kit contains a single-point calibrator. The calibrator, along with 0.9% saline as a zero reference, is used to generate a linear graph that will be used in the calculation of carbon dioxide concentrations in unknown samples.

   Diazyme Carbon Dioxide Control Set has controls for normal carbon dioxide level and abnormal carbon dioxide level. The controls are used as reference samples for checking the functionality of the Diazyme Carbon Dioxide Enzymatic Assay.

3. Special conditions for use statement(s):

   For professional use

4. Special instrument requirements:

   Any instrument with temperature control of 37 +/- 0.5°C that is capable of reading absorbance accurately at 405 or 415 nm. Performance of the assay was demonstrated on the Roche Diagnostics Cobas Mira instrument.

I. Device Description:

The Carbon Dioxide Enzymatic Assay kit contains of 5 x 20 mL of Reagent 1 and 1 x 2 mL of Calibrator. The reagents are supplied ready-to-use. Reagent 1 is composed

of phosphoenolpyruvate (PEP), phosphoenolpyruvate carboxylase (PEPC), NADH, and malate dehydrogenase (MDH) in buffer. The Calibrator is composed of 30 mM sodium bicarbonate in saline.

The Carbon Dioxide Controls kit contains 2 x 2 mL (one 25 mmol/L sodium bicarbonate in buffer and one 40 mmol/L sodium biocarbonate in buffer). The controls are supplied ready-to-use.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Carbon Dioxide-L3K Assay

2. Predicate 510(k) number(s):

   k990754

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Indications for Use	Quantitative determination of carbon dioxide	Same
Principle	Enzymatic assay involving phosphoenolpyruvate carboxylase (PEPC) and malate dehydrogenase (MDH) with spectrophotometric determination of carbon dioxide	Same

Differences		
Item	Device	Predicate
Specimen(s)	Human serum or plasma	Human serum
Reportable Range	1.12-50 mmol/L	2.9-50 mmol/L

K. Standard/Guidance Document Referenced (if applicable):

Clinical Laboratory Standards Institute EP5-A

Clinical Laboratory Standards Institute EP6-A
Clinical Laboratory Standards Institute EP7-A
Clinical Laboratory Standards Institute EP9-A

L. Test Principle:

Carbon dioxide is determined spectrophotometrically based on two coupled enzyme reactions including phosphoenolpyruvate carboxylase (PEPC) and malate dehydrogenase (MDH). PEPC catalyzes the first reaction and produces oxaloacetate. In the presence of MDH, the reduced cofactor is oxidized by oxaloacetate. This results in a decrease in absorbance at 405 or 415 nm that is directly proportional to carbon dioxide concentration in the sample.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

The precision at normal and abnormal high levels was evaluated on the Cobas Mira instrument according to CLSI EP5-A. In the study, two specimens containing 25 mM and 40 mM CO_2 were tested with two runs per day with duplicates over 20 working days. The results are below.

Within Run Precision

	25 mM CO_2	40 mM CO_2
Number of data points	80	80
Mean (mM)	24.1	40.1
SD (mM)	0.56	0.91
CV	2.3%	2.3%

Run to Run Precision

	25 mM CO_2	40 mM CO_2
Number of data points	80	80
Mean (mM)	24.1	40.1
SD (mM)	0.68	1.32
CV	2.8%	3.3%

The precision of the Diazyme Carbon Dioxide Enzymatic Assay was also evaluated on the Cobas Mira instrument with samples in the abnormal low range. In the study, twenty specimens containing approximately 16 mM CO_2 were tested in three separate runs over two days. The concentrations ranged between 15.39 – 16.96 mM CO_2. The results are below.

Number of data points	60
Mean (mM)	16.1
SD (mM)	0.27
CV	1.7%

b. Linearity/assay reportable range:

Two protocols were followed to demonstrate linearity. In Protocol A, six levels of commercial Casco CO_2 standard ranging from 0 to 50 mM were tested in triplicate on the Cobas Mira automated analyzer. In Protocol B, eleven levels of linearity set prepared by diluting a serum control containing 50 mM CO_2 with saline (according to CLSI EP6-A) were tested. Protocol A yielded recoveries ranging from approximately 99% to 105%. Protocol B yielded recoveries ranging from approximately 90% to 106%.

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

The calibrator and controls are prepared by diluting sodium bicarbonate solution with saline to the target value(s).

Value assignment of the calibrator on the Cobas Mira instrument consisted of the following. The prepared lot of calibrator was tested in 10 runs on the instrument using a commercially available assay and a primary CO_2 reference standard with traceability to NIST 351 and NIST 924 Carbonate Standards. The mean value of the calibrator and %CV were calculated from replicate analysis.

The same lot of calibrator was tested in 10 runs on the instrument using the Diazyme Carbon Dioxide Enzyme Assay, the same primary CO_2 reference standard, and two patient serum samples previously tested with the commercially available device. The mean value of the calibrator and the %CV were calculated from replicate analysis.

Value assignment of the controls on the Cobas Mira instrument consisted of the following. One lot of the Level 1 and Level 2 controls, along with the primary CO_2 reference standard and two patient serum samples previously tested with a commercially available assay, were tested with 5 runs on the Cobas Mira using one lot of the assay. The mean values and ranges of the controls were calculated.

To assess reagent and calibrator stability, three lots of the assay kits with three lots of calibrators after opening were tested. The results demonstrated that the reagents and calibrators are stable for about 12 days at 37ºC and for at least 10 weeks at 25ºC. The shelf-life of the assay kit is at least 12 months at 2-8ºC.

The real time stability study is ongoing.

To assess open bottle stability of the controls, three lots of Diazyme Carbon Dioxide Controls were tested. The results demonstrated that the reagents and calibrators are stable for about 12 days at 37ºC and for at least 12 weeks at 25ºC. The shelf-life of the assay kit is at least 12 months at 2-8ºC. The real time stability study is ongoing.

d. Detection limit:

To demonstrate limit of detection (LOD), a 1 mM CO_2 sample was tested in 12 replicates on the Cobas Mira. The LOD, defined as the mean + 3SD, is 1.12 mM CO_2.

e. Analytical specificity:

To determine the level of interference from substances normally present in serum, the device was tested with 25 mM and 40 mM CO_2 serum samples spiked with varying concentrations (according to CLSI EP7-A). Five levels for each substance were tested in triplicate. Less than 10% deviation from 25 mM and 40 mM was observed when tested up to the concentrations below.

Interfering Substance	**Concentration**
Triglyceride	1000 mg/dL
Ascorbic Acid	5 mg/dL
Bilirubin	40 mg/dL
Bilirubin Conjugate	40 mg/dL
Hemoglobin	1000 mg/dL

f. Assay cut-off:

Not applicable.

2. Comparison studies:

a. Method comparison with predicate device:

Performance of the Diazyme Carbon Dioxide Enzymatic Assay was compared with performance of a commercially available assay on a Cobas Mira analyzer. Sixty serum (consisting of pooled and individual patient samples) and sixty individual patient plasma samples were analyzed. Some samples were spiked with stock solution of CO_2 to target concentrations, to ensure distribution across the reportable range. Serum samples ranging from 5.9 to 44.5 mmol/L gave a correlation coefficient of 0.9859. Linear regression analysis resulted in the following equation: $y = 1.0447x – 0.9742$. Plasma

samples ranging from 5.85 to 40.64 mmol/L gave a correlation coefficient of 0.9731. Linear regression analysis resulted in the following equation: y = 0.9863x + 0.1486.

b. Matrix comparison:

Not applicable

3. Clinical studies:

a. Clinical Sensitivity:

Not applicable

b. Clinical specificity:

Not applicable

c. Other clinical supportive data (when a. and b. are not applicable):

The functionality of the device was evaluated at an independent outside laboratory. In each run, a low control serum sample containing 25 mM carbon dioxide and a high control serum sample containing 40 mM carbon dioxide were analyzed on their Cobas Mira instrument. The results were as follows:

Low Control Serum Sample			**High Control Serum Sample**		
25	24	24	40	40	39
Average = 24			Average = 40		

4. Clinical cut-off:

Not applicable

5. Expected values/Reference range:

Normal values of CO_2 in serum or plasma were based on literature. The sponsor recommends that each laboratory establish an expected range characteristic for the local population.

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.